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Association of soluble interleukin-2 receptor alpha with laboratory parameters and clinical findings of hemophagocytic lymphohistiocytosis patients: The first report from South of Iran

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Abstract:

INTRODUCTION: Hemophagocytic lymphohistiocytosis (HLH) is caused by overactivation of immune system. Gene mutations, infections, malignant, and autoimmune trigger the development of the disease.

MATERIALS AND METHODS: Clinical data and peripheral blood samples of 21 patients suspected of HLH were collected in Shiraz Medical Centers 2017–2018. Peripheral blood samples were analyzed for soluble interleukin-2 receptor alpha (sIL2Rα) marker (sCD25), and the results were compared with 36 normal controls as well as comparison with clinical findings and other laboratory parameters.

RESULTS: Twenty-one patients (11 males and 10 females) with an average age of 5.2 were investigated. In this study, peripheral blood samples were taken from 16 newly diagnosed patients before treatment, and five were posttreatment blood samples. The mean sIL2Rα level before treatment in 16 patients was 9023 pg/ml. The mean peripheral blood sample of the 36 controls was 3025 pg/ml. The mean of the five posttreatment samples was 4198 pg/ml. Significant difference between pretreatment and the control group was observed. However, no significant difference was detected between after treatment samples and the control group. By comparing the sIL2Rα levels between patients with increased aspartate aminotransaminase (AST) and patients with normal AST level, there was a significant difference in the amount of IL2Rα level.

CONCLUSION: This study highlights the importance of IL2Rα marker in the diagnosis and follow-up, during treatment and suppression. Furthermore, a significant difference with respect to AST level requires further investigation.

Keywords:

Hemophagocytic lymphohistiocytosis, soluble CD25, soluble interleukin-2 receptor alpha

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Introduction

Hemophagocytic lymphohistiocytosis (HLH) is an invasive and potentially life-threatening syndrome, caused by the uncontrolled and persistent activation of cytotoxic T-lymphocytes and NK cells. This overactivation of immune system also leads to increased secretion of inflammatory cytokines, activation of macrophages and systemic inflammatory signs and symptoms. What separates this disease from other inflammatory conditions is the high amount of pathological immune-related inflammation that leads to life-threatening damage to several organs. It often affects

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infants from birth to 18 months of age, but it is seen in children and adults.[1]

Clinically, HLH is often subdivided as primary/familial, in which there is a predisposing genetic defect in immune function. As a secondary or reactive, in which there is no genetic defect, it is generally due to an infectious agent, malignancy and/or autoimmunity. In the secondary cases, the search for the underlying agent and its proper management is critical for its treatment.[2]

This disease is a diagnostic challenge, as there are no clinical signs or pathognomonic laboratory findings, and symptoms are often nonspecific. Symptoms include fever, pancytopenia, increased ferritin, multiple organ dysfunction, and pathologic findings of hemophagocytosis.[1] In the first stage, fever is a good sign of infection, or the laboratory findings of pancytopenia, even with fever can have more common differential diagnoses, especially in the pediatric age group such as acute leukemia.[3]

The HLH-2004 diagnostic protocol added the first cytokine receptor biomarker family of soluble interleukin-2 receptor (sIl2r or sCD25, the alpha chain of the IL2 receptor complex) to the diagnostic criteria. In HLH-2004, the sIL2R criteria refers to ≥2400 U/ml. HLH-2004 introduced eight criteria for diagnosing HLH, of which five should be present, if the patient has no related genetic defect. In addition to IL2Rα criteria, other criteria are cytopenia, fever, splenomegaly, high ferritin, hypertriglyceridemia or hypofibrinogenemia, evidence of hemophagocytosis in bone marrow, spleen, or lymph nodes, and low or absent NK cell activity.[4]

The interleukin-2 receptor has three components such as alpha, beta, and gamma. The beta and gamma subunits are fundamentally active, but the alpha subunit is expressed only after cell activation. These IL2Rα or CD25 or Tac antigens together with IL2rβ or CD122 form a heterodimeric messenger molecule that affects the cell cycle through tyrosine kinase phosphorylation in JAK1 and JAK3. The soluble interleukin-2 receptor is a shortened protein of 40–45 kDa, which is dissociated from the 55 kDa protein IL2Rα when activated by T-cells. For this reason, this marker indicates the activation of T-cells.[5,6]

In the present study, the aim was to evaluate the level of IL2Rα in HLH disease at the time of diagnosis, after treatment and also to obtain epidemiological, clinical, and laboratory information about the disease at affiliated hospitals with Shiraz University of Medical Sciences (SUMS), Southern Iran, 2017–2018.

Materials and Methods

This study was conducted among clinically suspected HLH patients in Shiraz Medical Centers between 2017 and 2018, having 3–5 of six criteria of fever, organomegaly, cytopenia, high ferritin, high triglyceride or low fibrinogen, hemophagocytosis in tissue (HLH) as inclusion criteria. Not having the inclusion criteria or patient's unwillingness to participate was considered as exclusion criteria. After explaining the study objectives, informed consent was obtained from the patient's parents. The patient's information was extracted from their hospital records.

The collected data are as follows: gender, age at onset of the disease, grade of fever, and other associated symptoms. Complete blood count (CBC) parameters, fibrinogen and ferritin levels, organomegaly including splenomegaly and hepatomegaly, aspartate aminotransaminase (AST) level, history of immunosuppression, treatment initiation date, outcome of the patient and initiation factor if it is detected for secondary HLH. Bone marrow results were also recorded. This study was approved by the Local Ethics Committee of SUMS.

Soluble interleukin-2 receptor alpha(CD25) marker assay

Sampling

After selecting eligible individuals, the peripheral blood sample was prepared (without anticoagulant). After centrifugation in a maximum of 1 h, the serum samples were collected and stored at −70°C.

Peripheral blood samples were divided into two groups. Sixteen samples were taken at the time of diagnosis before treatment and five posttreatment samples, which we did not have pretreatment samples. As the control group, 36 persons, age and sex matched, without any disease at the time of sampling came for checkup to Motahari clinic were selected and after informed consent enrolled in the study.

Method

Serum assay was performed by the sandwich enzyme-linked immunosorbant assay (ELISA) using Diaclone Cd25 ELISA kit (Germany). Calculation of test results: using a standard curve based on the specific concentration of standard solutions and their optical readings, concentrations of other specimens were determined. Concentrations were measured in units of pg/ml.

Statistical analysis of data

The data were collected from clinical records, and the results of peripheral blood sCD25 test were entered into the SPSS 25 software (SPSS, Inc., Chicago, IL, USA). Kolmogorov–Smirnov, Pearson's correlation test,

independent *t*-test, analysis of variance (ANOVA), and Chi-square tests were used for data analysis.

Results

Finally, 21 patients were included in this study. Eleven patients (52.4%) were male and 10 (47.6%) were female. The mean age of patients at the time of diagnosis was 5.2 years, in a range from 2 months to 33 years old. All patients had fever at the onset of symptoms. The recorded temperature was >38.5°C, and two patients (9.52%) had a temperature >39.4°C.

Associated symptoms

Associated symptoms in addition to fever at the time of referral included jaundice in 4 patients (19.04%), rash in 4 patients (19.04%), and nausea and vomiting, diarrhea, constipation, edema, and polyneuropathy in one patient, each one equivalent to 4.76%.

A total of 18 patients (85.71%) had organomegaly, of which 11 had hepatosplenomegaly (61.1%), five had splenomegaly, and two had hepatomegaly.

The laboratory findings of the patients are summarized in Table 1.

Hemophagocytosis was observed in 38.1% of the cases in bone marrow aspiration smears [Figure 1].

Soluble CD25 (IL2Rα) results: in the 16 samples taken at the time of diagnosis, the lowest IL2Rα level was 3160 pg/ml, and the highest level was 12510 pg/ml, with a mean of 9023 pg/ml. Serum levels of this marker were evaluated in all 36 controls. The Kolmogorov–Smirnov method confirmed the normality of the data distribution. Then, the independent *t*-test was used to compare the levels of marker in the two groups of patients and the controls ($P < 0.001$) [Table 2].

Table 1: Laboratory findings of patients

Parameters	Lower limit (mg/dl)	Upper limit (mg/dl)	Mean (mg/dl)
WBC/mcL	980	193,00	4487
ANC/mcL	60	6170	1582
HB g/dl	2.1	11.2	7.6
PLT/mcL	6000	328,000	62,237
Triglyceride mg/dl	143	4025	603.8
Fibrinogen mg/dl	106	484	199.8
Ferritin ng/ml	695	40,000	12,563

WBC=White blood cell, ANC=Absolute neutrophil count, PLT=Platelet

In 16 patients who were tested for IL2Rα pretreatment, the association of this marker with other clinical information was assessed using Pearson's test, independent *t*-test, and one-way ANOVA. No correlation was found between IL2Rα level, age, white blood cell (WBC) count, neutrophil count, Hb, platelet count, triglyceride, fibrinogen, and ferritin ($P > 0.05$). Furthermore, IL2Rα level was not significantly different between the two groups, with and without organomegaly, with and without hemophagocytosis, living and dead, and in four groups according to the number of cytopenia. In this study, based on the amount of liver enzyme AST, 16 patients were divided into two groups: normal and increased. Whit independent *t*-test, IL2Rα level in group with increased AST enzyme and group with normal AST was significantly different ($P = 0.02$).

Discussion

HLH is a rare and life-threatening disease that might be primary with gene defect or secondary, due to infections, rheumatologic diseases or malignancies, especially the hematologic ones. In this disorder, there is immune dysregulation that causes increased activation of cytotoxic T-cells, macrophages, and increased amount of some interleukins and cytokines, such as IL2Rα that was entered in 2004 diagnostic criteria of the disease called HLH-2004 criteria.[1]

By entering patients suspected based on HLH-2004 criteria without considering IL2Rα level, 21 patients were finally evaluated.

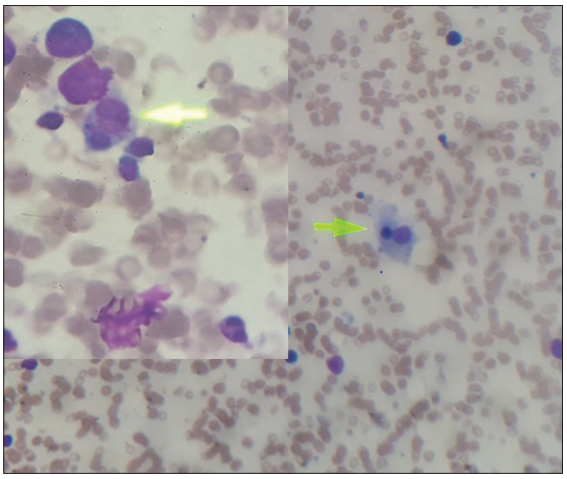

Figure 1: Hemophagocytosis in bone marrow aspiration of two patients in our study (Wright stain, ×400, Right. Oil Emerssion, Upper Left)

Table 2: The results of soluble interleukin-2 receptor

sIL2 Rα	Lowest level (pg/ml)	Highest level (pg/ml)	Mean (pg/ml)	*P*
Control (*n*=36)	928	7107	3025 (SD 1620)	Control versus pretreatment <0.001
Pretreatment (*n*=16)	3160	12,510	9023	Posttreatment versus pretreatment <0.05
Posttreatment (*n*=5)	2006	8298	4198	Control versus posttreatment >0.05

sIL2Rα=Soluble interleukin-2 receptor alpha, SD=Standard deviation

All patients in this study had fever at baseline, and the temperature of all patients was >38.5°C, which was consistent with the criteria of HLH-2004 presented by Henter *et al*. Among other associated symptoms, the most common were jaundice and rash, which were the common symptoms reported by Henter *et al*.[4]

Among the CBC parameters included total WBC count, neutrophil count, hemoglobin and platelet count, the mean of two hemoglobin and platelet parameters was similar to HLH-2004 cutoff criterion, hemoglobin <9, and platelets <100,000, which was in line with Chen's *et al*. study.[7]

Among other laboratory parameters, triglycerides and ferritin means were similar to those of the HLH-2004 cutoff criteria, and the mean fibrinogen was higher than the cutoff criteria. This is in contrast to Chen's *et al*. study, where the mean triglyceride was below the cutoff's criteria level, and the average fibrinogen was the same as the cutoff's criteria. In both our and Chen's *et al*.'s study, the mean ferritin was at the cutoff level of HLH-2004 criteria.[7] Based on this criteria only, splenomegaly was counted as organomegaly criteria, and in our study, 76% of patients showed splenomegaly, which is in line with the mentioned criteria.

Only 38% of patients' bone marrow specimens showed hemophagocytosis, but according to Henter *et al*.'s study, repeated bone marrow examination, as well as search for hemophagocytosis in other organs such as the lymph node, spleen, and liver, can be helpful.[4]

In a new criteria developed by Fardet *et al*., an increase in liver enzymes AST >30 IU/L is rated, which is not the case in HLH criteria 2004,[8] whereas 42.8% of our patients had this increase.

There was a significant difference between the levels of IL2Rα at the time of diagnosis in HLH patients compared to the control group. The mean serum level of IL2Rα was not significantly different in the five patients who were in remission when they were compared with the control group. However, a good comparison could not be made due to the unavailability of patients' serum levels before the treatment.

Studying the IL2Rα marker for the diagnosis of HLH and predicting the response to treatment and disease activity has been the subject of several studies. The first study of IL2Rα in 1989 by Komp *et al*. on nine children with hemophagocytic syndrome showed a significant increase in IL2Rα levels in untreated patients (23,600–72,500 units/ml).[9] In the United States in 2013, Mellor-Heineke *et al*. study of ten children with HLH found that all patients showed a decrease in serum IL2Rα levels during treatment.[10] A study by Gao *et al*. 2015, in China, investigated 22 HLH children showed a well correlation of IL2Rα levels with disease activity, and during the acute phase of the disease, these values were higher than the normal population (mean: 2493.89 pg/ml in pretreatment patients compared to 993.09 pg/ml in normal population). Furthermore, these amounts declined after effective treatment with clinical improvement (mean: 1557.10 pg/ml 2 weeks after treatment).[11]

In a study by Hayden *et al*. 2017, Canada, on 38 adult cases with HLH, it was found that the 2400 units/ml limit for IL2Rα included in 2004 criteria was 100% sensitive and 63% specific for HLH detection. Moreover, high values of more than 10,000 units/ml confirm the disease in adults with 93% specificity.[12] In a 2018 study in South Korea by Yoon *et al*. on 55 adult patients over 18 years of age, among other cytokines only, IL2Rα cytokine was significantly elevated (2856 units/ml vs. 1098 units/ml). This increase was seen, especially in patients with poor response to steroids.[13]

As noted in the above studies, elevated serum level of IL2Rα is important in the diagnosis of HLH, which can be used to monitor treatment and to evaluate disease activity.

In our study, the association between IL2Rα levels and clinical and laboratory findings was also assessed among 16 patients, whose IL2Rα level were assessed at baseline; however, according to statistical tests, correlation of IL2Rα level with age, WBC count, neutrophils, Hb, platelets, triglycerides, fibrinogen, and ferritin was not observed. Furthermore, IL2Rα level in two different genders, in the two groups with and without organomegaly, with and without hemophagocytosis, living and dead, and also in four groups according to the number of cytopenia were not significantly different. The association between the outcome and IL2Rα level is contrary to the results of Imashuku's *et al*. studies, 1991 and 1995[14,15] and also in contrast to Zhang's *et al*., 2011.[2] However, no significant difference was observed with other parameters similar to Imashuku's *et al*. findings in the year 1991 study, which reported the lack of association with ferritin levels,[14] and similar to Zhang's *et al*. study, no association was found with hemophagocytosis in bone marrow, splenomegaly, cytopenia, and ferritin levels.[2] Our results for ferritin were in contrast to the results of Bleesing *et al*.[16] that showed a correlation with ferritin although their population had a macrophage activation syndrome, which none of our patients were evaluated for.

Of note, in the present study, the significant difference between IL2Rα levels in the group with elevated AST

and the group without elevated AST, requires further investigation, even though two studies investigated it for the diagnosis of HLH patients without any direct comparison with the amount of IL2Rα.[8,17]

There are several challenges to find the etiology of this syndrome. In our study, only three patients, leishmaniasis, as the underlying causative agent was determined and classified as secondary HLH. Concerning Calaazar in a 2015 study by Blázquez-Gamero *et al*. in Spain, ten pediatric patients of 24 with visceral leishmania showed symptoms of hemophagocytic syndrome, and the leishmania parasite was the most common protozoan trigger of secondary HLH.[18]

One case, which had three HLH 2004 criteria and suspected HLH, malignancy was eventually found to be the underlying cause and diagnosed with ALL Type T. In the US, 2015, Berliner and Thotova stated that ALL is one of the most common malignant diseases associated with HLH, and some clinical symptoms of both are similar; hence, timely diagnosis is important given the different nature and treatment of the two diseases, as a major challenge.[19] The rest of patients (80%) had unknown etiology and their primary and secondary etiologies were unclear. The reason for this increase in uncertainties in our population can be due to limitations for precise identification of viral factors, such as PCR and the transient viral factors that lead to the undetection of traces. These patients also need to be screened for gene mutation to identify familial cases. However, in a study by Yasumi *et al*., a number of routine laboratory markers, such as LDH and peripheral blood lymphocytes as well as sIL2R/ferritin ratio were used to determine the probable etiology of this disease, which should be further studied and considered.[20]

Finally, regarding the patient criteria, this study is based on HLH-2004 criteria, which are currently the most internationally accepted criteria. It has to be mentioned that regardless of IL2Rα level and NK cell activity status, which is not routinely performed in our centers, of the 21 patients, 20 (95.23%) had at least five criteria. In only one patient with only three criteria, even after IL2Rα measurement, the criteria still remained three, and the patient did not fulfill the HLH 2004 criteria, and after bone marrow examination, the patient was diagnosed with T-cell ALL.

Conclusion

Serum levels of sIL2Rα marker in HLH patients at the time of diagnosis increased significantly compared to healthy controls. After treatment and during remission, these values were not significantly different from those of the normal population. This study only showed the association between serum level of CD25 and elevated AST, and no significant relationship was found between serum level of this marker and other laboratory and clinical parameters.

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Conflicts of interest

There are no conflicts of interest.

References

1. Chandrakasan S, Filipovich AH. Hemophagocytic lymphohistiocytosis: Advances in pathophysiology, diagnosis, and treatment. J Pediatr 2013;163:1253-9.
2. Zhang L, Zhang S, Xu J, Hu Y, Wang L, Zhang X, *et al*. Significance of soluble interleukin-2 receptor in patients with hemophagocytic lymphohistiocytosis. Leuk Lymphoma 2011;52:1360-2.
3. Safaei A, Shokripour M, Omidifar N. Bone marrow and karyotype findings of patients with pancytopenia in Southern Iran. Iran J Med Sci 2014;39:333-40.
4. Henter JI, Horne A, Aricó M, Egeler RM, Filipovich AH, Imashuku S, *et al*. HLH-2004: Diagnostic and therapeutic guidelines for hemophagocytic lymphohistiocytosis. Pediatr Blood Cancer 2007;48:124-31.
5. Rivière S, Galicier L, Coppo P, Marzac C, Aumont C, Lambotte O, *et al*. Reactive hemophagocytic syndrome in adults: A retrospective analysis of 162 patients. Am J Med 2014;127:1118-25.
6. Emile JF, Abla O, Fraitag S, Horne A, Haroche J, Donadieu J, *et al*. Revised classification of histiocytoses and neoplasms of the macrophage-dendritic cell lineages. Blood 2016;127:2672-81.
7. Chen Y, Wang Z, Luo Z, Zhao N, Yang S, Tang Y. Comparison of Th1/Th2 cytokine profiles between primary and secondary haemophagocytic lymphohistiocytosis. Ital J Pediatr 2016;42:50.
8. Fardet L, Galicier L, Lambotte O, Marzac C, Aumont C, Chahwan D, *et al*. Development and validation of the HScore, a score for the diagnosis of reactive hemophagocytic syndrome. Arthritis Rheumatol 2014;66:2613-20.
9. Komp DM, McNamara J, Buckley P. Elevated soluble interleukin-2 receptor in childhood hemophagocytic histiocytic syndromes. Blood 1989;73:2128-32.
10. Mellor-Heineke S, Villanueva J, Jordan MB, Marsh R, Zhang K, Bleesing JJ, *et al*. Elevated granzyme b in cytotoxic lymphocytes is a signature of immune activation in hemophagocytic lymphohistiocytosis. Front Immunol 2013;4:72.
11. Gao ZY, Li XY, Bhandari V, Li LD, Lan D. Pre-B-cell colony-enhancing factor is markedly elevated in childhood hemophagocytic lymphohistiocytosis. Genet Mol Res 2015;14:5287-95.
12. Hayden A, Lin M, Park S, Pudek M, Schneider M, Jordan MB, *et al*. Soluble interleukin-2 receptor is a sensitive diagnostic test in adult HLH. Blood Adv 2017;1:2529-34.
13. Yoon JH, Park KH, Yoon SY, Min GJ, Park SS, Jeon YW, *et al*. Natural-killer cell cytotoxicity and interleukin-2R as a relevant marker for diagnosis of secondary hemophagocytic lymphohistiocytosis in adult patients: The results of prospective

Phase II observational study. Am Soc Hematology 2018; 132 (Suppl 1) 4939.
14. Imashuku S, Ikushima S, Esumi N, Todo S, Saito M. Serum levels of interferon-gamma, cytotoxic factor and soluble interleukin-2 receptor in childhood hemophagocytic syndromes. Leuk Lymphoma 1991;3:287-92.
15. Imashuku S, Hibi S, Sako M, Ishida Y, Mugishima H, Chen J, *et al*. Soluble interleukin-2 receptor: A useful prognostic factor for patients with hemophagocytic lymphohistiocytosis. Blood 1995;86:4706-7.
16. Bleesing J, Prada A, Siegel DM, Villanueva J, Olson J, Ilowite NT, *et al*. The diagnostic significance of soluble CD163 and soluble interleukin-2 receptor alpha-chain in macrophage activation syndrome and untreated new-onset systemic juvenile idiopathic arthritis. Arthritis Rheum 2007;56:965-71.
17. Debaugnies F, Mahadeb B, Ferster A, Meuleman N, Rozen L, Demulder A, *et al*. Performances of the H-score for diagnosis of hemophagocytic lymphohistiocytosis in adult and pediatric patients. Am J Clin Pathol 2016;145:862-70.
18. Blázquez-Gamero D, Domínguez-Pinilla N, Chicharro C, Negreira S, Galán P, Pérez-Gorricho B, *et al*. Hemophagocytic lymphohistiocytosis in children with visceral leishmaniasis. Pediatr Infect Dis J 2015;34:667-9.
19. Tothova Z, Berliner N. hemophagocytic syndrome and critical illness: New insights into diagnosis and management. J Intensive Care Med 2015;30:401-12.
20. Yasumi T, Hori M, Hiejima E, Shibata H, Izawa K, Oda H, *et al*. Laboratory parameters identify familial haemophagocytic lymphohistiocytosis from other forms of paediatric haemophagocytosis. Br J Haematol 2015;170:532-8.